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(54) **ATTENUATED CHICKEN-POX VIRUS OKA STRAIN GENE 62 AND METHOD FOR IDENTIFYING VIRUS STRAIN FOR ATTENUATED LIVE CHICKEN-POX VACCINE BY USING THE GENE**

(57) The present application provides the gene 62 of Oka vaccine virus, which comprises at least with the following base substitutions (a) to (d) in the nucleotide sequence of SEQ ID No. 1: (a) the base A at position 2110 is G; (b) the base A at position 3100 is G; (c) the base T at position 3818 is C; and (d) the base A at position 4006 is G, and a method for identifying the Oka vaccine virus and an attenuated varicella-zoster virus strain acceptable as virus strain for live attenuated varicella vaccine, by using the gene 62.

Description

Technical Field

5 [0001] The present application relates to gene 62 of the Oka vaccine virus and a method for identifying a virus strain for live attenuated varicella-zoster vaccine (referred to as "attenuated varicella-zoster virus strain" hereinafter) using the DNA sequence of gene 62. More specifically, the application relates to the gene 62 for use in discriminating the Oka vaccine virus and an attenuated varicella-zoster virus strain acceptable as the effective component of live attenuated vaccine for varicella-zoster, from the wild-type Oka vaccine virus (referred to as "virulent parental strain" or
10 "parental strain" hereinafter) and other wild-type varicella-zoster virus strains; and a method for identifying an attenuated varicella-zoster virus, based on the nucleotide sequence of the gene 62.

Background Art

15 [0002] Varicella-zoster virus (abbreviated as VZV; referred to as "varicella virus" or "VZV" hereinafter) is the pathogenic virus of varicella and zoster in humans. Virus derived from "Oka vaccine virus" (Japanese Patent Publication No. 41202/1978; Lancet 2: 1288-1290, 1974) as one attenuated varicella-zoster virus strain has been used as the single unique seed for preparing live attenuated varicella vaccines, which are widely used worldwide [Requirements for Varicella Vaccine (Live) adopted 1984: WHO Technical Report Series, No. 725, pp 102-124, 1985].

20 [0003] Currently, the safety and effectiveness of the live attenuated varicella vaccines is certificated by the regulation of the passage number of the varicella seed virus with approval of the preparation (seed lot system). More specifically, so as to avoid the risk of genetic modification of the attenuated virus over continuous passages of the seed virus so that the resulting virus resumes the pathogenicity, it is defined under regulation that under provision that the passage number of the seed when approved is designated 0, virus within the total passage number of 10 from the passage
25 number 0 is essentially used for such vaccines. In other words, the quality control and quality certificate of the live attenuated varicella vaccines depend on the observance of the seed lot system by each vaccine manufacture.

[0004] Meanwhile, no clinical reaction emerges in healthy children after the inoculation of the live attenuated varicella vaccines. The onset of varicella or zoster only rarely occurs in inoculated subjects with disorders of the immune function. However, whether or not such clinical reaction is due to the vaccination or spontaneous infection with wild-type
30 varicella virus has never been identified. Thus, it is important epidemiologically to analyze the differentiation of the Oka vaccine virus as the seed virus of the live attenuated varicella vaccine from other wild-type VZV strains. Hence, a number of methods for the analysis have been proposed. Since the structure of the VZV genomic genes was reported (Journal of General Virology, 67: 1759-1816, 1986), methods have been proposed, for example, on the basis of the difference in DNA nucleotide sequence among VZV strains (Journal of Virology, 59: 660-668, 1986), the presence or
35 absence of restriction enzyme *Pst*I site (Japanese Journal of Experimental Medicine, 59: 233-237, 1989), determination based on RFLP (restriction fragment length polymorphism) by PCR (polymerase chain reaction) (Journal of Virology, 66: 1016-1020, 1992), and a combination of the presence or absence of the *Pst*I site and the RFLP analysis of PCR products (Journal of Clinical Microbiology, 33:658-660, 1995). However, these methods are at low reliability when used singly. Thus, it has been difficult to definitely differentiate the Oka vaccine virus from other wild-type VZV strains.

40 [0005] Alternatively, the applicants of the present application have made diverse gene analysis of the Oka vaccine virus and other varicella virus strains (for example, known wild-type strains and wild-type strains newly isolated from patients with spontaneously infected varicella, and the like), and have identified eight requirements to differentiate the two kinds of strain types. Further the applicants have found that an unknown virus is acceptable as live varicella vaccine only when the virus satisfies all the eight requirements, and they have made a patent application based on the finding
45 (WO 97/43420; referred to as "previous invention" hereinafter).

[0006] The method of the previous invention can discriminate the Oka vaccine virus from other varicella-zoster virus strains, but can never discriminate the Oka vaccine virus from the parental strain. Because all the eight required indicators are essentially examined, disadvantageously, the method needs a lot of time and labors until the discrimination is established.

50 [0007] The inventors of the present application have made investigations about a method for possibly differentiating the Oka vaccine virus from the parental strain, and they have found that the Oka vaccine virus, the parental strain and other wild-type varicella virus strains can be differentiated from each other in a simple and reliable manner, by detecting the mutation of varicella virus gene 62.

[0008] The invention of the present application has been attained on the basis of such novel finding, and a purpose
55 of the invention is to provide the Oka vaccine virus-specific gene 62.

[0009] It is an additional purpose of the invention to provide the protein encoded by the gene 62 and a peptide thereof, an antibody and cytotoxic T lymphocyte (abbreviated as CTL) prepared by using them as antigen, and an attenuated varicella-zoster virus strain carrying the gene 62.

[0010] It is a still additional purpose of the invention to provide a method for identifying an attenuated varicella-zoster virus strain using the presence or absence of the mutation of the gene 62 as an indicator, and a live attenuated varicella vaccine prepared by using the attenuated virus strain identified by the method as the seed virus.

5 Disclosure of Invention

[0011] The present application provides the following individual inventions to satisfy the aforementioned purposes.

10 (1) Gene 62 of Oka vaccine virus, which comprises at least the following base substitutions (a) to (d) in the nucleotide sequence of SEQ ID No. 1:

- (a) the base A at position 2110 is G;
- (b) the base A at position 3100 is G;
- (c) the base T at position 3818 is C; and
- 15 (d) the base A at position 4006 is G.

(2) The gene 62 of the Oka vaccine virus of the above (1), which comprises at least one or more of the following base substitutions (e) to (g) in addition to the base substitutions (a) to (d):

- 20 (e) the base A at position 1251 is G;
- (f) the base A at position 2226 is G; and
- (g) the base A at position 3657 is G.

25 (3) The gene 62 of the Oka vaccine virus of the above (2), which comprises at least one or more of the following base substitutions (h) to (o), in addition to the base substitutions (a) to (d) and at least one or more of the base substitutions (e) to (g):

- (h) the base T at position 162 is C;
- (i) the base T at position 225 is C;
- 30 (j) the base T at position 523 is C;
- (k) the base T at position 1565 is C;
- (l) the base T at position 1763 is C;
- (m) the base T at position 2652 is C;
- (n) the base T at position 4052 is C; and
- 35 (o) the base T at position 4193 is C.

(4) A fragment of DNA encoding the gene 62 of any one of above (1) to (3), which comprising 10 or more base pairs including the base-substituted portions.

40 (5) A protein encoded by the gene 62 of the Oka vaccine virus of above (1), which comprises at least the following amino acid substitutions (A) to (D) in the amino acid sequence of SEQ ID No. 1:

- (A) the amino acid residue Ser at position 628 is Gly;
- (B) the amino acid residue Arg at position 958 is Gly;
- 45 (C) the amino acid residue Val at position 1197 is Ala; and
- (D) the amino acid residue Ile at position 1260 is Val.

6. The protein of the above (5), which comprises at least one or more of the following amino acid substitutions (E) to (H) in addition to the amino acid substitutions (A) to (D):

- 50 (E) the amino acid residue Met at position 99 is substituted with Thr;
- (F) the amino acid residue Len at position 446 is Pro;
- (G) the amino acid residue Val at position 512 is Ala; and
- (H) the amino acid residue Leu at position 1275 is Ser.

55 (7) A peptide as a part of the protein of the above (5) or (6), which comprises 5 or more amino acid residues including the amino acid-substituted portions.

(8) An antibody or a cytotoxic T lymphocyte prepared by using the protein of the above (5) or (6) or the peptide of the above (7) as an antigen.

(9) An attenuated varicella-zoster virus strain, of which genomic gene 62 is the gene 62 of any one of the above (1) to (3).

(10) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus with the same base substitutions in the gene 62 as the gene 62 of any one of the above (1) to (3).

(11) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus carrying genomic DNA to which the DNA fragment of the above (4) hybridizes.

(12) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus producing an antigen recognized by the antibody or the cytotoxic T lymphocyte of the above (8).

(13) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising digesting a DNA fragment comprising at least from 2107th to 2229th in the nucleotide sequence of SEQ ID No. 1 by using restriction enzymes *NaeI* and *BssHII*, and selecting a varicella-zoster virus from which the DNA fragment is digested into 2 or 3 fragments.

(14) A method according to the above (13), wherein the DNA sequence is prepared by PCR using the oligonucleotides of the nucleotide sequence of SEQ ID No. 2 and 3 as primers and varicella-zoster virus gene 62 as a template.

(15) An attenuated varicella-zoster virus strain identified by a method of any one of the above (10) to (14).

(16) A live attenuated vaccine for varicella-zoster prepared by using the attenuated varicella-zoster virus strain of the above (9) or (15) as a seed virus.

Brief Description of Drawings

[0012]

Fig. 1, upper column depicts the schematic view of the structure of varicella virus gene 62; Fig. 1, lower column collectively depicts the sequencing results of the 9 clones derived from the gene 62 of Oka vaccine virus and the sequencing results of the gene 62 of the virulent parental strain.

Fig. 2 is the agarose gel electrophoresis charts of the RFLP results of the PCR products of the gene 62 of each of the following varicella-zoster virus strains: Lane 1 for the Oka vaccine virus; Lane 2 for the highly toxic parental strain; Lane 3 for Kawaguchi strain; Lanes 4 to 8 for varicella-derived strain; Lanes 9 to 13 for zoster-derived strain; the lanes on both the sides are for size markers.

Fig. 3 depicts the results of CAT assay to test transactivation activity of gene 62 products (IE62) of the Oka vaccine virus and virulent Oka parental strain on individual promoters; Fig. 3A shows that IE62s expressed from the effector plasmids transactivate the gene 4 (IE4) promoter in a dose-dependent fashion, wherein the CV1 cell is cotransfected with pCAT-IE4 at a constant amount (0.25µg) and pVac-G62 (open square) or pPar-G62 (closed circle) at various amounts; and Fig. 3B, C, D, E and F show the activities of IEG2s on the individual promoters of the gene 62 coding for IE62, the gene 28 for DNA polymerase, the gene 29 for major DNA binding protein (abbreviated as MDBP), the gene 14 for glycoprotein C (abbreviated as gC) and the gene 68 for gE, respectively.

Best Mode for Carrying Out the Invention

[0013] Varicella virus gene 62 is one of the gene responsible for the transactivation function (function for activating transcription in the trans-form) and locates in the region of the base numbers 105142 to 109242 in the whole sequence of the varicella virus genome (Journal of General Virology, 67: 1759-1816, 1986). As shown in the upper column of Fig. 1, a transcription starts from the transcription start site along the arrow direction, so the nucleotide strand of the base numbers 109242 to 105142 in the whole genome sequence serves as "+" strand in the gene 62. Thus, the first base "A" in the SEQ ID No. 1 corresponds to the 5' terminal base (base at position 109242 in the whole genome sequence)

of the + strand. In the following description, furthermore, base number is sometimes described as the base number in the whole genome sequence. Because the sequence of the gene 62 in the whole genome sequence is "-" strand, however, the gene 62 is complementary to the nucleotides shown in SEQ ID No. 1.

5 [0014] The gene 62 of Oka vaccine virus of the invention is a gene with the base substitutions (a) to (d) shown in Table 1 in the nucleotide sequence of SEQ ID No. 1. Otherwise, the gene 62 of Oka vaccine virus is a gene with at least one or more of the base substitutions (e) to (g) in Table 1 in addition to the base substitutions (a) to (d), or the gene additionally with at least one or more of the base substitutions (h) to (o) in Table 1.

10 [0015] The protein of the attenuated varicella virus of the invention is encoded by the gene 62, wherein the protein is with the amino acid substitutions (A) to (D) shown in Table 1 in the amino acid sequence of SEQ ID No. 1. Otherwise, the protein is with the amino acid substitutions of at least one or more of (E) to (H) shown in Table 1, in addition to the amino acid substitutions (A) to (D).

Table 1

Base substitutions	Amino acid substitutions
(a) 2110th A → G	(A) 661st Ser → Gly
(b) 3100th A → G	(B) 958th Arg → Gly
(c) 3818th T → C	(C) 1197th Val → Ala
(d) 4006th A → G	(D) 1260th Ile → Val
(e) 1251st A → G	
(f) 2226th A → G	
(g) 3657th A → G	
(h) 162th T → C	
(i) 225th T → C	
(j) 523rd T → C	(E) 99th Met → Thr
(k) 1565th T → C	(F) 446th Leu → Pro
(l) 1763rd T → C	(G) 512nd Val → Ala
(m) 2652nd T → C	
(n) 4052nd T → C	(H) 1275th Leu → Ser
(o) 4193rd T → C	

40 [0016] It is additionally suggested that the Oka vaccine virus carrying the gene 62 with the base substitutions causing such amino acid substitutions is attenuated probably due to the overall reduction of the expression of a protein required for the formation of virus particle because of the defection or attenuation of the transactivator function.

45 [0017] Such gene 62 of Oka vaccine virus can be isolated for example from the known Oka vaccine virus (BIKEN Lot-65: The Research Foundation of Microbial Diseases of Osaka University or ATCC VR-795) in a conventional manner. Otherwise, such Oka vaccine virus gene 62 can be isolated from an attenuated varicella-zoster virus strain identified by a method as described below.

50 [0018] The fragment of DNA of the present invention is that encodes the gene 62 set forth above, and comprises 10 or more base pairs including the base substitutions. The DNA fragment can be prepared by cleaving the genomic DNA of Oka vaccine virus or an attenuated varicella-zoster virus strain newly identified by the inventive method with appropriate restriction enzymes. The DNA fragment can be used for example as DNA probe for differentiating the Oka vaccine virus or the attenuated varicella-zoster virus strain from the highly toxic parental strain or other wild-type varicella-zoster virus strains.

55 [0019] Additionally, the protein of the present invention can be prepared by expressing the gene 62 in microorganisms for example *Escherichia coli* or yeast. More specifically, a DNA fragment carrying the coding region of the gene 62 is inserted in an expression vector with an origin replicable in microorganisms, a promoter, a ribosome-binding site, a DNA-cloning sites and a terminator to prepare an expression vector, to thereby transform a host cell; by subsequently culturing the resulting transformant, the protein encoded by the gene 62 can be produced in microorganisms at a mass scale. Otherwise, the gene 62 can be expressed in the form of a fusion protein with other proteins. By cleaving the

resulting fusion protein with an appropriate protease, only the objective protein can be recovered.

[0020] These proteins can be used as antigens for preparing for example antibody and CTL.

[0021] The peptide of the present invention is of a sequence comprising 5 or more amino acid residues including the amino acid substitutions in the amino acid sequence of the protein. These peptides can be used as antigens for preparing antibodies and CTL.

[0022] The antibody of the present invention can be prepared in the form of polyclonal antibody or monoclonal antibody using the protein per se or a partial peptide thereof as an antigen by a known method. Additionally, the CTL of the present invention can be prepared by using the protein per se or a partial peptide thereof as an antigen by a known method. These antibodies and CTL can be used for the method for identifying a newly isolated attenuated varicella-zoster virus strain.

[0023] The attenuated varicella-zoster virus strain of the invention have the identical base substitutions compared with those of the gene 62 of Oka vaccine virus. Therefore, the attenuated varicella-zoster virus strain of the invention is acceptable as a virus strain for live attenuated varicella vaccine. Such new attenuated varicella-zoster virus strain can be identified by selecting a varicella virus carrying the base substitutions in gene 62. More specifically, such new attenuated varicella-zoster virus strain can be differentiated from the wild-type varicella-zoster virus strain by the method using for example the DNA fragment or the antibody or CTL.

[0024] Otherwise, the new attenuated varicella-zoster virus strain can be identified by the method proposed in this application, namely the method comprising cleaving a DNA sequence comprising at least from 2107th to 2229th in the nucleotide sequence of SEQ ID No. 1 by using restriction enzymes *NaeI* and *BssHII*, and selecting a varicella virus from which the DNA sequence is cleaved into 2 to 3 fragments. As has been described above, the base A at position 2110 in the gene 62 of Oka vaccine virus is substituted with G. Additionally, the base A at position 2226 is sometimes substituted with G. Consequently, the base substitution at position, an A vs. G, results in the creation of a restriction enzyme *NaeI* site (GCCGGC) at positions 2107 to 2112 in SEQ ID No. 1. Like that, the base substitution at position 2226, an A vs. G, makes *BssHII* site (GCGCGC) at positions 2224 to 2229 in SEQ ID No. 1. In case that the DNA fragment at least comprising the nucleotides from 2107th to 2229th in SEQ ID No. 1 is cleaved with the two restriction enzymes, hence, the DNA fragment is cleaved into 2 or 3 fragments when the DNA fragment is derived from the Oka vaccine virus or the attenuated varicella virus. In case that the DNA sequence is derived from a wild-type varicella virus or the virulent parental strain of the Oka vaccine virus, while, the DNA sequence is never cleaved by either restriction enzymes, and remains as a single fragment because of no presence of such base substitutions as described above. The DNA fragment cleaved by the restriction enzymes can be prepared by PCR using the oligonucleotides, the nucleotide sequence of SEQ ID No. 2 and No. 3, as primers and the varicella virus gene 62 as an identification subject as template.

[0025] The live attenuated varicella vaccine of the present invention is prepared by using the attenuated varicella-zoster virus strain newly identified by the method described above as the seed virus. Such live vaccine can be prepared in the same manner as the method for preparing conventional live attenuated varicella vaccines using the Oka vaccine virus as the seed virus.

Examples

[0026] The invention will now be described specifically in detail in the following Examples but the invention is never limited to the following examples.

Example 1

[0027] The nucleotide sequence of the gene 62 of Oka vaccine virus and the gene 62 of the highly toxic parental strain were determined. The Oka vaccine virus (live vaccine strain), Biken Lot-65: The research Foundation for Microbial Diseases of Osaka University was used. The virulent parental strain (wild-type Oka vaccine virus) was propagated in MRC-5 cells, and was then used.

[0028] According to the method described in the Example 1 of the previous invention (WO 97/43420), more specifically, genomic DNA were extracted from the Oka vaccine virus and the virulent parental strain, and each DNA was amplified by PCR using primers designed by referring to the nucleotide sequence of the Dumas strain (J. Gen. Virol., 67: 1759-1816, 1986). The entire gene 62 was amplified as three entire overlapping pieces using three sets of primer pair (sense primers G62N1, G62N2 and G62N3 and antisense primers G62R1, G62R2 and G62R3) as shown in Table 2. Each sense primer had M13 forward (-38) sequence (nucleotides at positions 1 to 19 in G62N1-3) at their 5' end, and each antisense primer had M13 reverse sequence (nucleotides at positions 1 to 20 in G62R1-3) linked at their 5' end.

Table 2

Primers	Sequence	Genomic Position
G62N	5'-CCGAGCTCGAATT/GTAGATTCATAAAAACCGTTCCGC-3'	105103-105139
<i>Xba</i> I cleavage site		
G62N1	5'-TTTCCCAGTCACGACGTTGTTTCATAAAAACCGTTCCGC-3'	105121-105139
G62N2	5'-TTTCCCAGTCACGACGTTGCAGGCACAACCGGTTACTCAG-3'	106455-106475
G62N3	5'-TTTCCCAGTCACGACGTTGTTTGGTCTTACGAATCCTCGG-3'	107844-107864
G62R	5'-ACCTGATCAGAATTCTGCA/GAGCGGTCTCTCCTTAAACGC-3'	109381-109362
<i>Pst</i> I cleavage site		
G62R1	5'-GGATAACAATTTACACAGGTTCTGATCATCTACGATCCG-3'	106600-106581
G62R2	5'-GGATAACAATTTACACAGGCAAATTCGGATGATTCGGAC-3'	107950-107931
G62R3	5'-GGATAACAATTTACACAGGAGCGGTCTCTCCTTAAACGC-3'	109381-109362

[0029] Each PCR reaction consisted of 200 mM of deoxynucleotide triphosphate [each], 0.3 μ M of each primer, an extremely low amount of template DNA, and 2.5 U of Ex Taq (manufactured by TaKaRa) in 50 μ l of Ex Taq buffer. One set of primer pairs (SEQ ID Nos. 3 and 8) included 6 % DMSO in the reaction mixture. Amplification was carried out at 30 cycles of denaturation (94 °C for one minute), annealing (55 °C for 1.5 minutes) and extension (72 °C for 2 minutes), using thermal cycler (Perkin-Elmer, USA).

[0030] All PCR products were purified using the PCR purification kit (QIAGEN GmbH, Germany) to remove primers. Direct sequence of the purified PCR products was performed with a sequencing kit (Amersham, Co., UK) using M13 forward primer or reverse primer labeled with a fluorescent dye IRO-40, analyzed using a DNA sequencer Model 4000L (LI-COR Co., USA).

[0031] By the same method, the DNA sequence of genes 4, 14 and 61 of Oka vaccine virus and the virulent parental strain were determined.

[0032] Consequently, there were no nucleotide change in the genes 4, 14 and 61 between the Oka vaccine virus and the virulent parental strain. As shown in Table 3, 15 bases differences were found in the gene 62 between Oka vaccine virus and the virulent parental strain. Every base substitutions was a T vs C or an A vs G. At eight positions, the nucleotide changes found in the vaccine virus resulted from mixture of two kinds of nucleotide at a single position (R in Table 3). From the respect of 9 bases, additionally, the gene 62 of each of the Oka vaccine virus and the virulent parental strain was different from the gene 62 of the Dumas strain.

[0033] Based on the aforementioned results, it was confirmed that the attenuated Oka vaccine virus could be differentiated from the virulent parental strain in terms of the sequence difference of gene 62.

Table 3

Genomic Position	Vaccine Strain	Parental Strain	Dumas Strain
105169	R (noncoding)	A (noncoding)	A
105310	R (Ser/Leu)	A (Leu)	A
105356	C (Val)	T (Ile)	T
105451	G	G	A
105512	C	C	A
105544	G (Ala)	A (Ala)	A
105705	C (Ala)	T (Ala)	T
106262	C (Gly)	T (Arg)	T
106710	R (Ala)	A (Ala)	A
107136	C (Ala)	T (Ala)	T

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Table 3 (continued)

	Genomic Position	Vaccine Strain	Parental Strain	Dumas Strain
5	107165	T	T	C
	107252	C (Gly)	T (Ser)	T
	107303	C	C	T
	107599	R (Ala/Val)	A (Val)	A
10	107607	A	A	C
	107715	C	C	T
	107797	R (Pro/Leu)	A (Leu)	A
	108111	C (Pro)	T (Pro)	T
15	108747	G	G	A
	108838	R (Thr/Met)	A (Met)	A
	108951	A	A	G
20	109044	G	G	C
	109137	R (silent)	A (silent)	A
	109200	R (silent)	A (silent)	A

Example 2

[0034] The entire gene 62 was cloned from Oka vaccine virus and the virulent parental strain by PCR.

[0035] PCR conditions were same as in Example 1 except for the use of the oligonucleotides G62N and G62R in Table 1 as primer pairs, the addition of 6 % DMSO in the reaction mixture, and an extension time of 6 minutes for every cycle. The full length of each gene 62 was amplified by PCR.

[0036] Because the primers individually had restriction enzymes *Xba*I and *Pst*I sites, the purified PCR product was digested with these restriction enzymes. And then the digested product was inserted into pUC19, which had been digested in advance with the corresponding enzymes. After the transformation of *E. coli* JM109 with hybrid plasmids, 9 plasmids were extracted from the Oka vaccine and the virulent parental transformants for DNA sequencing.

[0037] The results are shown in Fig. 1. As shown in Fig. 1, plural bases among the bases at 15 positions in each of the 9 clones of the Oka vaccine virus were different from those of the virulent parental strain. Particularly, the 7 bases at base positions 1251, 2110, 2226, 3100, 3667, 3818 and 4006 in all of the clones were different from the bases at the same positions in the parental strain. No nucleotide changes were observed in any of the 9 clones from the virulent parental strains for these 15 sites.

Example 3

[0038] Partial sequences of the gene 62s of various varicella-zoster virus strains were analyzed by RFLP.

[0039] The Oka vaccine virus and the virulent parental strain were used as way in Example 1. Additionally, the Kawaguchi strain, one of wild-type varicella-zoster virus strains and 10 samples of wild-type virus strains isolated from vesicular fluids from non-vaccinated 5 patients with varicella and 5 patients with zoster were used. Herein, the Kawaguchi strain and the wild-type varicella-zoster virus strains isolated from these patients were propagated in HEL cell or MRC-5 cell over 2 to 3 times, and were then used.

[0040] The gene 62 of each varicella virus was amplified by PCR using the oligonucleotides of SEQ ID Nos. 2 and 3 as primers. The oligonucleotide of SEQ ID No. 2 was the reverse primer and corresponding to the bases at positions 1846 to 1863 in the varicella virus gene 62 (SEQ ID No. 1), while the oligonucleotide of SEQ ID No. 3 was the forward primer corresponding to the bases at positions 2609 to 2620 thereof. PCR conditions were the same as in Example 1, except that the extension time was one minute for every cycle. Three microliters of each PCR product were digested sequentially with 4 U *Nae*I (TaKaRa) at 37 °C for 1.5 hours and 4U *Bss*HI (TaKaRa) at 50 °C for 1.5 hours. The digested DNA fragments were analyzed by electrophoresis on 4 % agarose gel (NuSieve 3:1, FMC BioProducts, Co.) with ethidium bromide staining.

[0041] The results are shown in Fig. 2. As apparently shown in Fig. 2, the PCR product (Lane 1) of the gene 62 from Oka vaccine virus was cleaved into three fragments (402 bp, 264 bp and 116 bp) by *Nae*I and *Bss*HI, whereas the PCR

products (Lanes 2-13) of the virulent parental strain and the wild-type virus strains isolated from the patients were never digested by either restriction enzymes and remained as a single fragment.

[0042] The aforementioned results establish the confirmation that by cleaving a DNA sequence at least comprising the base at position 2107 to 2229 in the varicella virus gene 62 with restriction nucleases *NaeI* and *BssHII*, a varicella virus with a DNA sequence thereby cleaved into 2 to 3 fragments could be identified as the Oka vaccine virus or a virus strain acceptable as attenuated varicella virus.

Example 4

[0043] So as to analyze the correlation between the eight amino acid mutations and the transactivation activities of the gene 62 products, IE62, chloramphenicol transferase (CAT) assays were performed in cells cotransfected with a reporter plasmid having a promoter sequence of each VZV gene upstream of CAT gene and an effector plasmid carrying the gene 62 of Oka vaccine virus or the highly toxic parental strain.

1. Materials and Methods

1.1 Plasmids

[0044] Reporter plasmids pIE4-CAT, pgC-CAT, pPOL-CAT, pMDBP-CAT, pIE62-CAT and pgE-CAT contained about 750 bases upstream of the initiation codon the promoter region of genes 4, 14, 28, 29, 62 and 68, respectively. Each promoter region was amplified from the Oka vaccine strain by PCR using specific primers which had *NheI*- or *BglII* site at the 5' end. Conditions of PCR were the same as in Example 1, except for the extension time of one minute. The amplified products were digested with *NheI* and *BglII*, and were then inserted into upstream of CAT gene of pCAT3-Basic plasmid (Promega, Co., USA).

[0045] Effector plasmid pPar-G62 was selected among 9 clones from gene 62 clone of the virulent parental strain. Clone 9 of Fig. 1 (the clone containing 13 base substitutions and 8 amino acid substitutions) was selected from among the 9 clones from the Oka vaccine strain and used as the effector plasmid, pVac-G62.

1.2 Transfection

[0046] CV1 cells were cultured in a 35-mm plastic dish at 10^5 cells/dish and were transfected by lipofection using Superfect (QIAGEN GmbH). Each reaction mixture for transfection consisted of 0.25 µg of reporter plasmid and various amounts (0-1 µg) of either effector plasmid. The total DNA amount in each transfection was kept constant at 2.5 µg by adding the vector pUC19. All experiments were repeated at least three times, with independent DNA transfection.

1.3 CAT assay

[0047] Forty four hours after transfection, the total protein and CAT levels in the cell were assayed. The cell was washed three times with phosphate-buffered saline, and then lysed in lysis buffer (Boehringer Mannheim Corporation, Germany). The CAT concentration of the individual lysate was determined by a CAT ELISA kit (Boehringer Mannheim Corporation, Germany) and was standardized to each protein concentration, that was determined by the Bio-Rad protein reagent (Bio-Rad, USA).

2. Results

[0048] All of the VZV gene promoters tested displayed very low-level basal activity; little or no CAT expression was observed when no effector plasmid was transfected.

[0049] The gene 4 promoter derived from Oka strain was transactivated by both gene 62 products (IE62s) of the virulent parental strain and the Oka strain (Fig. 3A). The transactivation activity of IE62 of the virulent parental strain was stronger than that of the IE62 by the Oka vaccine strain. When the lowest dose (0.25 µg) of the effector plasmids was transfected, the activity of the virulent parental strain IE62 was 7.8-fold higher than that by the Oka strain IE62. As the effector concentration was increased, the difference in the activity of the two IE62 proteins diminished.

[0050] Little CAT could be detected in the cells transfected with the reporter plasmid pCAT-IE62 (Fig. 3B).

[0051] Furthermore, pCAT-Pol and pCAT-MDBP carrying the VZV genes 28 and 29 promoters, respectively (Fig. 3C and 3D), and pCAT-gE carrying the promoter sequence of the gene 68 (Fig. 3F) were activated by both IE62s from the virulent parental strain and the Oka vaccine strain. Each dose-dependent curve was similar to the pCAT-IE4 response, and the activity of the virulent parental strain IE62 was higher than that of the Oka vaccine strain IE62 at all dose. At the lowest dose of effector plasmids, in particular, the activity of the virulent parental strain IE62 was 7.6-fold, 5.6-fold and

1.8-fold higher than that of the Oka vaccine strain IE62. Little CAT activity was detected in the cell transfected with pCAT-gC carrying the promoter region of the gene 14 (Fig. 3E).

5 [0052] The aforementioned results establish the confirmation that the expression product (IE62) of the VZV gene 62 activated the promoters of the pre-early gene 4 (ORF4), early gene 28 (DNA polymerase: Pol) and gene 29 (major DNA binding protein: MDBP) and delayed gene 68 (glycoprotein: gE). It is additionally of more importance that the transcription activity of the Oka vaccine strain IE62 was constantly lower than that of the highly toxic parental strain IE62. In other words, the mutations in the gene 62 of the Oka vaccine strain have some influence on virus replication, which induces the attenuation of VZV.

10 Industrial Applicability

[0053] In accordance with the present invention, the Oka vaccine virus or a varicella-zoster virus strain acceptable as attenuated varicella vaccine can be accurately discriminated in a simple manner from other wild-type varicella viruses and the highly toxic parental strain of the Oka vaccine virus. In accordance with the present invention, further-
15 more, attenuated varicella virus antigens for live vaccine can be prepared at a mass scale. Owing to the inventions, vaccine preparation and the safety and effectiveness of vaccination can be achieved in a more reliable manner.

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Sequence Listing

5 <110> The Research Foundation for Microbial Diseases of Osaka University

<120> Gene 62 of Oka vaccine virus and method for identifying virus strain for live attenuated vaccine virus using the gene 62.

10 <140> PCT/JP99/05476

<141> 1999-10-05

<150> JP11-48964

15 <151> 1999-02-25

<160> 3

<210> 1

20 <211> 4226

<212> DNA

<213> Varicella-Zoster virus

25 <220>

<221> CDS

<222> 229..4158

30 <400> 1

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35 ACACGTTTTA AGTACTGTTG GAACTCCCTC ACCAACCGCA ATCGCAATCC TTTGAAGGCT 180

GCGAGAGCGT TTGGAAAAC T CGGGTACGTC TAAATTCACC CCAGTGCG ATG GAT 234

40 Met Asp

1

ACG CCG CCG ATG CAG CGC TCT ACA CCC GAA CGC GCG GGG TCG CCT GAT 282

45 Thr Pro Pro Met Gln Arg Ser Thr Pro Gln Arg Ala Gly Ser Pro Asp

5 10 15

ACT TTG GAG TTA ATG GAC CTG TTG GAC GCG GCC GCC GCG GCC GCC GAA 330

50 Thr Leu Glu Leu Met Asp Leu Leu Asp Ala Ala Ala Ala Ala Ala Glu

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	20	25	30	
5	CAC AGG GCC CGG GTG GTC ACC TCG AGT CAG CCT GAG GAT CTA CTA TTT	378		
	His Arg Ala Arg Val Val Thr Ser Ser Gln Pro Asp Asp Leu Leu Phe			
	35	40	45	50
10	GGA GAG AAC GGG GTC ATG GTG GGA CGG GAA CAT GAG ATC GTT TCA ATT	426		
	Gly Glu Asn Gly Val Met Val Gly Arg Glu His Glu Ile Val Ser Ile			
	55	60	65	
15	CCC TCC GTA TCG GGA CTT CAA CCA GAA CCC AGA ACG GAA GAT GTT GGC	474		
	Pro Ser Val Ser Gly Leu Gln Pro Glu Pro Arg Thr Glu Asp Val Gly			
	70	75	80	
20	GAA GAG CTA ACA CAA GAC GAC TAC GTA TGC GAG GAC GGT CAG GAT CTA	521		
	Glu Glu Leu Thr Gln Asp Asp Tyr Val Cys Glu Asp Gly Gln Asp Leu			
	85	90	95	
25	ATG GGC TCG CCT GTA ATC CCG CTG GCC GAG GTC TTC CAC ACC CGA TTC	570		
	Met Gly Ser Pro Val Ile Pro Leu Ala Glu Val Phe His Thr Arg Phe			
	100	105	110	
30	TCG GAG GCC GGC GCG CGA GAA CCA ACA GGA GCC GAT CGC TCC CTC GAG	618		
	Ser Glu Ala Gly Ala Arg Glu Pro Thr Gly Ala Asp Arg Ser Leu Glu			
	115	120	125	130
35	ACA GTC TCT CTC GGA ACG AAG CTT GCT AGG TCT CCA AAA CCA CCG ATG	666		
	Thr Val Ser Leu Gly Thr Lys Leu Ala Arg Ser Pro Lys Pro Pro Met			
	135	140	145	
40	AAC GAT GGG GAA ACG GGC AGA GGT ACG ACC CCT CCG TTC CCG CAG GCC	714		
	Asn Asp Gly Glu Thr Gly Arg Gly Thr Thr Pro Pro Phe Pro Gln Ala			
	150	155	160	
45	TTC TCC CCT GTA TCC CCC GCG TCT CCT GTT GGA GAC GCC GCC GGG AAC	762		
	Phe Ser Pro Val Ser Pro Ala Ser Pro Val Gly Asp Ala Ala Gly Asn			
	165	170	175	
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	GAT CAA CGG GAA GAC CAG CGG TCT ATA CCC CGA CAA ACG ACG AGA GGA	810
5	Asp Gln Arg Glu Asp Gln Arg Ser Ile Pro Arg Gln Thr Thr Arg Gly	
	180 185 190	
	AAT TCA CCA GGT TTG CCG TCG GTG GTC CAT CGA GAC AGA CAA ACT CAG	858
10	Asn Ser Pro Gly Leu Pro Ser Val Val His Arg Asp Arg Gln Thr Gln	
	195 200 205 210	
	TCC ATC TCG GGT AAA AAG CCG GGC GAT GAG CAA GCG GGT CAT GCG CAT	906
15	Ser Ile Ser Gly Lys Lys Pro Gly Asp Glu Gln Ala Gly His Ala His	
	215 220 225	
	GCA TCG GGG GAC GGA GTA GTT CTC CAG AAA ACT CAA CGG CCC GGT CAG	954
20	Ala Ser Gly Asp Gly Val Val Leu Gln Lys Thr Gln Arg Pro Ala Gln	
	230 235 240	
	GGA AAG AGC CCG AAG AAA AAG ACT TTG AAG GTT AAG GTC CCA CTC CCG	1002
25	Gly Lys Ser Pro Lys Lys Lys Thr Leu Lys Val Lys Val Pro Leu Pro	
	245 250 255	
	GCG CGG AAA CCC GGT GGA CCT GTA CCC GGC CCG GTT GAG CAA TTG TAC	1050
30	Ala Arg Lys Pro Gly Gly Pro Val Pro Gly Pro Val Glu Gln Leu Tyr	
	260 265 270	
	CAC GTC CTT TCG GAC AGC GTT CCG GCT AAG GGG GCA AAG GCG GAC CTG	1098
35	His Val Leu Ser Asp Ser Val Pro Ala Lys Gly Ala Lys Ala Asp Leu	
	275 280 285 290	
	CCG TTT GAG ACC GAT GAT ACC CGC CCA AGG AAA CAT GAT GCC CGG GGT	1146
40	Pro Phe Glu Thr Asp Asp Thr Arg Pro Arg Lys His Asp Ala Arg Gly	
	295 300 305	
	ATA ACA CCT CGC GTC CCT GGA CGT TCG TCG GGG GGC AAA CCT AGA GCG	1194
	Ile Thr Pro Arg Val Pro Gly Arg Ser Ser Gly Gly Lys Pro Arg Ala	
50	310 315 320	
	TTT TTG GCC CTG CCG GGA AGA TCC CAC GCA CCA GAC CCG ATT GAG GAT	1242
55		

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Phe Leu Ala Leu Pro Gly Arg Ser His Ala Pro Asp Pro Ile Glu Asp
325 330 335
5 GAC AGC CCA GTG GAG AAA AAG CCA AAG AGT CGT GAG TTT GTT TCG TCT 1290
Asp Ser Pro Val Glu Lys Lys Pro Lys Ser Arg Glu Phe Val Ser Ser
10 340 345 350
TCA TCG TCT TCG TCG TCG TGG GGA TCG TCA TCG GAG GAT GAA GAC GAT 1338
Ser Ser Ser Ser Ser Ser Trp Gly Ser Ser Ser Glu Asp Glu Asp Asp
15 355 360 365 370
GAA CCC CGG CGC GTT TCG GTG GGA AGT GAA ACT ACA GGC AGC AGG TCG 1386
Glu Pro Arg Arg Val Ser Val Gly Ser Glu Thr Thr Gly Ser Arg Ser
20 375 380 385
GGA CGC GAA CAC GCC CCT TCG CCG TCA AAT TCG GAT GAT TCG GAC TCA 1434
Gly Arg Glu His Ala Pro Ser Pro Ser Asn Ser Asp Asp Ser Asp Ser
25 390 395 400
AAT GAT GGT GGG TCG ACG AAA CAA AAT ATC CAA CCG GGA TAT CGA TCC 1482
Asn Asp Gly Gly Ser Thr Lys Gln Asn Ile Gln Pro Gly Tyr Arg Ser
30 405 410 415
ATC AGC GGT CCC GAT CCG AGG ATT CGT AAG ACC AAA CGT CTT GCG GGG 1530
Ile Ser Gly Pro Asp Pro Arg Ile Arg Lys Thr Lys Arg Leu Ala Gly
420 425 430
40 GAA CCG GGG CGC CAG AGA GAG AAA TCA TTT TCC CTG CCG CGA TCC AGA 1578
Glu Pro Gly Arg Gln Arg Gln Lys Ser Phe Ser Leu Pro Arg Ser Arg
435 440 445 450
45 ACC CCG ATA ATT CCC CCG GTG TCG GGG CCG CTC ATG ATG CCC GAC GGA 1626
Thr Pro Ile Ile Pro Pro Val Ser Gly Pro Leu Met Met Pro Asp Gly
455 460 465
50 AGC CCT TGG CCC GGA TCG GCG CCC CTC CCA TCC AAC AGG GTG CGG TTT 1674
Ser Pro Trp Pro Gly Ser Ala Pro Leu Pro Ser Asn Arg Val Arg Phe
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	470	475	480	
5	GGA CCG TCC GGG GAG ACC AGA GAG GGT CAC TGG GAG GAT GAG GCT GTG	1722		
	Gly Pro Ser Gly Glu Thr Arg Glu Gly His Trp Glu Asp Glu Ala Val			
	485	490	495	
10	AGA GCG GCG CCG GCT CGT TAC GAG GCC TCA ACT GAA CCC GTG CCG CTT	1770		
	Arg Ala Ala Arg Ala Arg Tyr Glu Ala Ser Thr Glu Pro Val Pro Leu			
	500	505	510	
15	TAC GTG CCG GAG TTG GGA GAT CCG GCT AGA CAG TAC CGC GCG CTG ATT	1818		
	Tyr Val Pro Glu Leu Gly Asp Pro Ala Arg Gln Tyr Arg Ala Leu Ile			
	515	520	525	530
20	AAC CTG ATC TAC TGT CCA GAC AGA GAC CCT ATA GCA TGG CTC CAG AAC	1866		
	Asn Leu Ile Tyr Cys Pro Asp Arg Asp Pro Ile Ala Trp Leu Gln Asn			
	535	540	545	
25	CCC AAG CTG ACC GGT GTC AAC TCG GCC CTG AAC CAG TTC TAC CAA AAG	1914		
	Pro Lys Leu Thr Gly Val Asn Ser Ala Leu Asn Gln Phe Tyr Gln Lys			
	550	555	560	
30	CTG TTG CCA CCG GGA CCG GCG GGT ACC GCC GTT ACG GGG AGC GTA GCG	1962		
	Leu Leu Pro Pro Gly Arg Ala Gly Thr Ala Val Thr Gly Ser Val Ala			
	565	570	575	
35	TCT CCC GTT CCG CAT GTA GGC GAA GCC ATG GCC ACG GGG GAG GCC CTC	2010		
	Ser Pro Val Pro His Val Gly Glu Ala Met Ala Thr Gly Glu Ala Leu			
	580	585	590	
40	TGG GCT CTC CCC CAC GCG GCC GCG GCC GTG GCT ATG AGC CGT CGG TAC	2058		
	Trp Ala Leu Pro His Ala Ala Ala Ala Val Ala Met Ser Arg Arg Tyr			
	595	600	605	610
45	GAC CGG GCC CAA AAA CAC TTT ATC CTA CAG AGT CTC CGC AGA GCC TTT	2106		
	Asp Arg Ala Gln Lys His Phe Ile Leu Gln Ser Leu Arg Arg Ala Phe			
	615	620	625	
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	GCC AGC ATG GCA TAC CCC GAG GCA ACG GGC TCC AGT CCG GCG GCG CGG	2154
	Ala Ser Met Ala Tyr Pro Glu Ala Thr Gly Ser Ser Pro Ala Ala Arg	
5	630 635 640	
	ATC TCC CGC GGT CAC CCT TCT CCA ACA ACC CCG GCC ACA CAG ACT CCC	2202
10	Ile Ser Arg Gly His Pro Ser Pro Thr Thr Pro Ala Thr Gln Thr Pro	
	645 650 655	
	GAC CCT CAG CCG TCG GCC GCC GCA CGC TCT CTT TCT GTG TGT CCA CCG	2250
15	Asp Pro Gln Pro Ser Ala Ala Ala Arg Ser Leu Ser Val Cys Pro Pro	
	660 665 670	
	GAT GAT CGT TTA CGA ACT CCG CGC AAG CGC AAG TCC CAG CCA GTC GAG	2298
20	Asp Asp Arg Leu Arg Thr Pro Arg Lys Arg Lys Ser Gln Pro Val Glu	
	675 680 685 690	
	AGC AGA AGC CTC CTC GAC AAG ATT AGG GAG ACA CCC GTC GCG GAC GCC	2346
25	Ser Arg Ser Leu Leu Asp Lys Ile Arg Glu Thr Pro Val Ala Asp Ala	
	695 700 705	
	CGG GTT GCA GAC GAT CAT GTG GTT TCC AAG GCC AAG AGG CGG GTA TCC	2394
30	Arg Val Ala Asp Asp His Val Val Ser Lys Ala Lys Arg Arg Val Ser	
	710 715 720	
	GAG CCC GTG ACC ATC ACC TCG GGC CCT GTG GTG GAT CCC CCC GCC GTA	2442
35	Glu Pro Val Thr Ile Thr Ser Gly Pro Val Val Asp Pro Pro Ala Val	
	725 730 735	
	ATA ACG ATG CCA CTT GAC GGA CCG GCC CCA AAC GGG GGA TTT CGG CGT	2490
40	Ile Thr Met Pro Leu Asp Gly Pro Ala Pro Asn Gly Gly Phe Arg Arg	
	740 745 750	
	ATT CCC CGG GGG GCC CTG CAT ACC CCG GTC CCG TCG GAC CAG GCT CGC	2538
45	Ile Pro Arg Gly Ala Leu His Thr Pro Val Pro Ser Asp Gln Ala Arg	
	755 760 765 770	
	AAG GCG TAC TGT ACC CCC GAA ACC ATC GCC CGT CTG GTC GAC GAC CCA	2586
50		
55		

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	775 780 785	
5	TTG TTT CCC ACG GCC TGG CGC CCT GCG CTA AGC TTT GAT CCC GGC GCC	2634
	Leu Phe Pro Thr Ala Trp Arg Pro Ala Leu Ser Phe Asp Pro Gly Ala	
10	790 795 800	
	TTG GCG GAA ATC GCC GCT CGG CGT CCG GGC GGA GGA GAC CGA CGG TTT	2682
	Leu Ala Glu Ile Ala Ala Arg Arg Pro Gly Gly Gly Asp Arg Arg Phe	
15	805 810 815	
	GGT CCA CCC AGC GGA GTG GAG GCG CTG CGA CGG AGG TGC GCC TGG ATG	2730
	Gly Pro Pro Ser Gly Val Glu Ala Leu Arg Arg Arg Cys Ala Trp Met	
20	820 825 830	
	CGG CAG ATC CCA GAC CCG GAG GAT GTG AGG CTT CTG ATC ATC TAG GAT	2778
25	Arg Gln Ile Pro Asp Pro Glu Asp Val Arg Leu Leu Ile Ile Tyr Asp	
	835 840 845 850	
	CCG TTG CCC GGA GAG GAC ATC AAC GGC CCC CTC GAG AGC ACC CTC GCG	2826
30	Pro Leu Pro Gly Glu Asp Ile Asn Gly Pro Leu Glu Ser Thr Leu Ala	
	855 860 865	
	ACA GAT CCG GGA CCG TCA TGG AGT CCA TCC CGA GGG GGA CTG TCT GTG	2874
35	Thr Asp Pro Gly Pro Ser Trp Ser Pro Ser Arg Gly Gly Leu Ser Val	
	870 875 880	
40	GTC CTG GCA GCC CTG AGT AAC CGG TTG TGC CTG CCG AGC ACT CAT GCC	2922
	Val Leu Ala Ala Leu Ser Asn Arg Leu Cys Leu Pro Ser Thr His Ala	
	885 890 895	
45	TGG GCC GGG AAC TGG ACC GGC CCG CCG GAC GTG TCC GCT TTG AAC GCC	2970
	Trp Ala Gly Asn Trp Thr Gly Pro Pro Asp Val Ser Ala Leu Asn Ala	
	900 905 910	
50	CGG GGC GTT TTA TTA CTG TCG ACC CGA GAC CTG GCC TTT GCC GGG GCC	3018
	Arg Gly Val Leu Leu Leu Ser Thr Arg Asp Leu Ala Phe Ala Gly Ala	
55		

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	915	920	925	930	
5	GTC GAG TAT CTA GGC TCG CGG TTG GCC TCT GCC CGG CGC CGG TTG CTG	3066			
	Val Glu Tyr Leu Gly Ser Arg Leu Ala Ser Ala Arg Arg Arg Leu Leu				
		925	940	945	
10	GTG TTG GAC GCG GTG GCC CTC GAG AGG TGG CCC AGG GAT GGA CCC GCT	3114			
	Val Leu Asp Ala Val Ala Leu Glu Arg Trp Pro Arg Asp Gly Pro Ala				
		950	955	960	
15	TTG TCT CAG TAT CAC GTG TAC GTC CGG GCC CCG GCG CGA CCG GAC GCC	3162			
	Leu Ser Gln Tyr His Val Tyr Val Arg Ala Pro Ala Arg Pro Asp Ala				
		965	970	975	
20	CAG GCC GTC GTC CGA TGG CCA GAC TCG GCG GTC ACA GAA GGA CTC GCC	3210			
	Gln Ala Val Val Arg Trp Pro Asp Ser Ala Val Thr Glu Gly Leu Ala				
		980	985	990	
25	CGG GCC GTG TTT GCA TCG TCG CGC ACC TTT GGG CCA GCG AGT TTT GCT	3258			
	Arg Ala Val Phe Ala Ser Ser Arg Thr Phe Gly Pro Ala Ser Phe Ala				
30	995	1000	1005	1010	
	CGT ATC GAG ACT GCG TTT GCC AAC CTG TAC CCG GGC GAA CAA CCC CTG	3306			
	Arg Ile Glu Thr Ala Phe Ala Asn Leu Tyr Pro Gly Glu Gln Pro Leu				
35		1015	1020	1025	
	TGT TTG TGC CGC GGT GGG AAC GTC GCA TAC ACC GTG TGT ACC CGC GCG	3354			
	Cys Leu Cys Arg Gly Gly Asn Val Ala Tyr Thr Val Cys Thr Arg Ala				
40		1030	1035	1040	
	GGC CCC AAG ACC CGC GTC CCC CTG TCG CCC CGT GAA TAC CGG CAG TAC	3402			
	Gly Pro Lys Thr Arg Val Pro Leu Ser Pro Arg Glu Tyr Arg Gln Tyr				
45		1045	1050	1055	
	GTG CTG CCG GGT TTT GAC GGT TGC AAG GAC CTC GCG CGA CAG TCT CGG	3450			
50	Val Leu Pro Gly Phe Asp Gly Cys Lys Asp Leu Ala Arg Gln Ser Arg				
		1060	1065	1070	
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	GGT CTG GGG CTC GGG GCA GCC GAC TTT GTG GAC GAG GCG GCA CAT AGC	3498
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5	1075 1080 1085 1090	
	CAC CGC GCA GCA AAC CGA TGG GGC CTG GGT GCC GCG CTT CGA CCC GTC	3546
10	His Arg Ala Ala Asn Arg Trp Gly Leu Gly Ala Ala Leu Arg Pro Val	
	1095 1100 1105	
	TTC CTT CCC GAG GGA CGG AGA CCG GGG GCC GCC GGG CCG GAG GCC GGC	3594
15	Phe Leu Pro Glu Gly Arg Arg Pro Gly Ala Ala Gly Pro Glu Ala Gly	
	1110 1115 1120	
	GAC GTA CCC ACC TGG GCG AGG GTG TTT TGC CGC CAC GCC CTG CTG GAA	3642
20	Asp Val Pro Thr Trp Ala Arg Val Phe Cys Arg His Ala Leu Leu Glu	
	1125 1130 1135	
	CCG GAC CCT GCC GCA GAA CCA CTC GTG CTT CCA CCC GTG GCC GGT CGG	3690
25	Pro Asp Pro Ala Ala Glu Pro Leu Val Leu Pro Pro Val Ala Gly Arg	
	1140 1145 1150	
	TCG GTG GCG CTG TAT GCG TCG GCG GAC GAG GCT CGG AAT GCC CTC CCC	3738
30	Ser Val Ala Leu Tyr Ala Ser Ala Asp Glu Ala Arg Asn Ala Leu Pro	
	1155 1160 1165 1170	
	CCG ATT CCC AGA GTA ATG TGG CCG CCC GGT TTT GGG GCC GCG GAG ACG	3786
35	Pro Ile Pro Arg Val Met Trp Pro Pro Gly Phe Gly Ala Ala Glu Thr	
	1175 1180 1185	
40	GTG TTG GAG GGG AGC GAC GGA ACA CGG TTC GTG TTC GGA CAC CAC GGG	3834
	Val Leu Glu Gly Ser Asp Gly Thr Arg Phe Val Phe Gly His His Gly	
	1190 1195 1200	
45	GGC TCG GAA CGG CCG GCA GAA ACC CAG GCG GGG CGA CAG CGG CGC ACC	3882
	Gly Ser Glu Arg Pro Ala Glu Thr Gln Ala Gly Arg Gln Arg Arg Thr	
	1205 1210 1215	
50	GCA GAC GAC AGA GAA CAC GCT TTG GAG CCG GAC GAT TGG GAG GTG GGG	3930

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Ala Asp Asp Arg Glu His Ala Leu Glu Pro Asp Asp Trp Glu Val Gly

1220 1225 1230

5 TGT GAA GAC GCG TGG GAC AGC GAG GAG GGG GGC GGG GAC GAC GGG GAC 3978

Cys Glu Asp Ala Trp Asp Ser Glu Glu Gly Gly Gly Asp Asp Gly Asp

10 1235 1240 1245 1250

GCA CCG GGG TCA TCC TTT GGG GTG AGC ATC GTG TCG GTG GCC CCG GGT 4026

Ala Pro Gly Ser Ser Phe Gly Val Ser Ile Val Ser Val Ala Pro Gly

15 1255 1260 1265

GTG CTG CGA GAC CGC CGG GTG GGC TTG CGC CCG GCG GTC AAG GTG GAG 4074

Val Leu Arg Asp Arg Arg Val Gly Leu Arg Pro Ala Val Lys Val Glu

20 1270 1275 1280

CTG TTG TCC TCG TCC TCG TCC TCC GAG GAC GAG GAC GAT GTG TGG GGA 4122

Leu Leu Ser Ser Ser Ser Ser Ser Ser Glu Asp Glu Asp Asp Val Trp Gly

25 1285 1290 1295

GGG CGC GGG GGG AGG AGC CCC CCG CAG AGT CCG GGG TGACGGAGTC 4168

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<211> 18

40 <212> DNA

<213> Artificial Sequence

45 <400> 2

GATCAAAGCT TAGCGCAG 18

50 <210> 3

<211> 18

55

<212> DNA

<213> Artificial Sequence

<400> 3

CCTATAGCAT GGCTCCAG

18

Claims

1. Gene 62 of Oka vaccine virus, which comprises at least the following base substitutions (a) to (d) in the nucleotide sequence of SEQ ID No. 1:

- (a) the base A at position 2110 is G;
- (b) the base A at position 3100 is G;
- (c) the base T at position 3818 is C; and
- (d) the base A at position 4006 is G.

2. The gene 62 of the Oka vaccine virus of claim 1, which comprises at least one or more of the following base substitutions (e) to (g) in addition to the base substitutions (a) to (d):

- (e) the base A at position 1251 is G;
- (f) the base A at position 2226 is G; and
- (g) the base A at position 3657 is G.

3. The gene 62 of the Oka vaccine virus of claim 2, which comprises at least one or more of the following base substitutions (h) to (o), in addition to the base substitutions (a) to (d) and at least one or more of the base substitutions (e) to (g):

- (h) the base T at position 162 is C;
- (i) the base T at position 225 is C;
- (j) the base T at position 523 is C;
- (k) the base T at position 1565 is C;
- (l) the base T at position 1763 is C;
- (m) the base T at position 2652 is C;
- (n) the base T at position 4052 is C; and
- (o) the base T at position 4193 is C.

4. A fragment of DNA encoding the gene 62 of any one of claims 1 to 3, which comprising 10 or more base pairs including the base-substituted portions.

5. A protein encoded by the gene 62 of the Oka vaccine virus of claim 1, which comprises at least the following amino acid substitutions (A) to (D) in the amino acid sequence of SEQ ID No. 1:

- (A) the amino acid residue Ser at position 628 is Gly;
- (B) the amino acid residue Arg at position 958 is Gly;
- (C) the amino acid residue Val at position 1197 is Ala; and
- (D) the amino acid residue Ile at position 1260 is Val.

6. The protein of claim 5, which comprises at least one or more of the following amino acid substitutions (E) to (H) in addition to the amino acid substitutions (A) to (D):

- (E) the amino acid residue Met at position 99 is Thr;

(F) the amino acid residue Leu at position 446 is Pro;
 (G) the amino acid residue Val at position 512 is Ala; and
 (H) the amino acid residue Leu at position 1275 is Ser.

- 5 7. A peptide as a part of the protein of claim 5 or 6, which comprises 5 or more amino acid residues including the amino acid-substituted portions.
8. An antibody or a cytotoxic T lymphocyte prepared by using the protein of claim 5 or 6 or the peptide of claim 7 as an antigen.
- 10 9. An attenuated varicella-zoster virus strain, of which genomic gene 62 is the gene 62 of any one of claims 1 to 3.
10. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus with the same base substitutions in the gene 62 as the gene 62 of any one of claims 1 to 3.
- 15 11. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus carrying genomic DNA to which the DNA fragment of claim 4 hybridizes.
- 20 12. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus producing an antigen recognized by the antibody or the cytotoxic T lymphocyte of claim 8.
- 25 13. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising digesting a DNA fragment comprising at least from 2107th to 2229th in the nucleotide sequence of SEQ ID No. 1 by using restriction enzymes *NaeI* and *BssHII*, and selecting a varicella-zoster virus from which the DNA fragment is digested into 2 or 3 fragments.
14. A method according to claim 13, wherein the DNA sequence is prepared by PCR using the oligonucleotides of the nucleotide sequence of SEQ ID No. 2 and 3 as primers and varicella-zoster virus gene 62 as a template.
- 30 15. An attenuated varicella-zoster virus strain identified by a method of any one of claims 10 to 14.
16. A live attenuated vaccine for varicella-zoster prepared by using the attenuated varicella-zoster virus strain of claim 9 or 15 as a seed virus.

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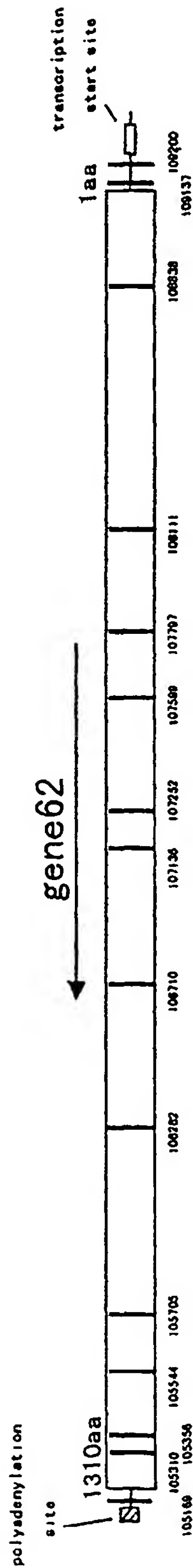
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Fig. 1



position		105169	105310	105356	105544	105705	106282	106710	107136	107232	107589	107797	108111	108338	108838	109137	109200
Oka parental	all clones	A	A	T	A	T	T	A	A	T	T	A	A	A	T	A	A
Oka vaccine	clone 1	A	A	C	G	C	C	G	G	C	C	A	C	A	C	A	G
	clone 2	A	A	C	G	C	C	A	A	C	C	A	C	A	C	A	A
	clone 3	G	G	C	G	C	C	A	A	C	C	A	C	A	C	A	G
	clone 4	A	G	C	G	C	C	G	G	C	C	A	C	A	C	A	G
	clone 5	G	A	C	G	C	C	A	A	C	C	A	C	A	C	A	A
	clone 6	A	A	C	G	C	C	T	A	C	C	A	C	A	C	A	G
	clone 7	G	A	C	G	C	C	A	A	C	C	A	C	A	C	A	A
	clone 8	G	A	C	G	C	C	A	A	C	C	A	C	A	C	A	A
	clone 9	G	G	C	G	C	C	A	A	C	C	A	C	G	G	G	A

*amino acid	-	Leu	Ile	Ala	Ala	Ala	Arg	Ala	Ala	Ala	Ser	Val	Leu	Pro	Met	-	-
	-	Ser	Val	Ala	Ala	Ala	Gly	Ala	Ala	Ala	Gly	Ala	Pro	Pro	Thr	-	-

Fig. 2

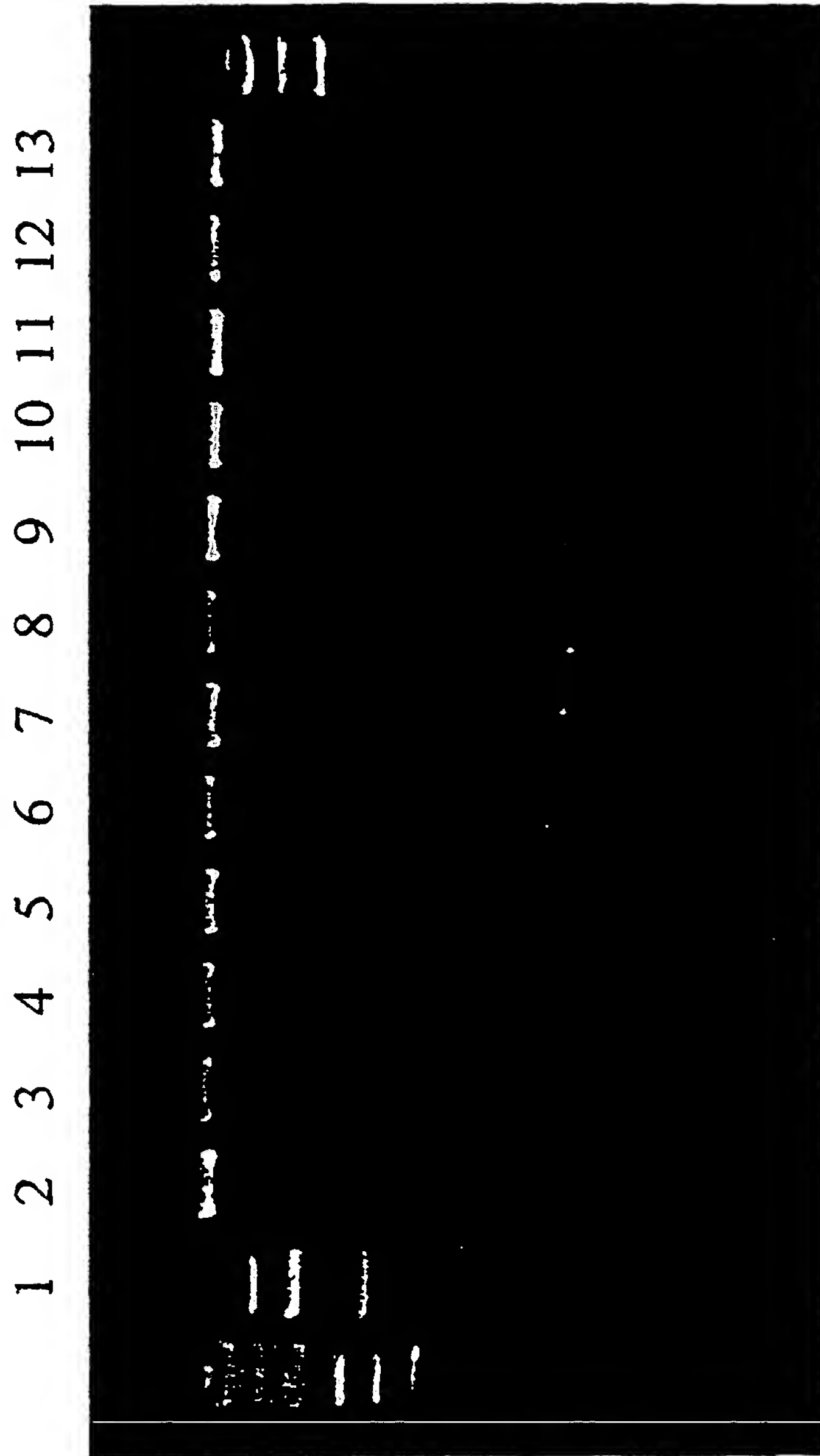
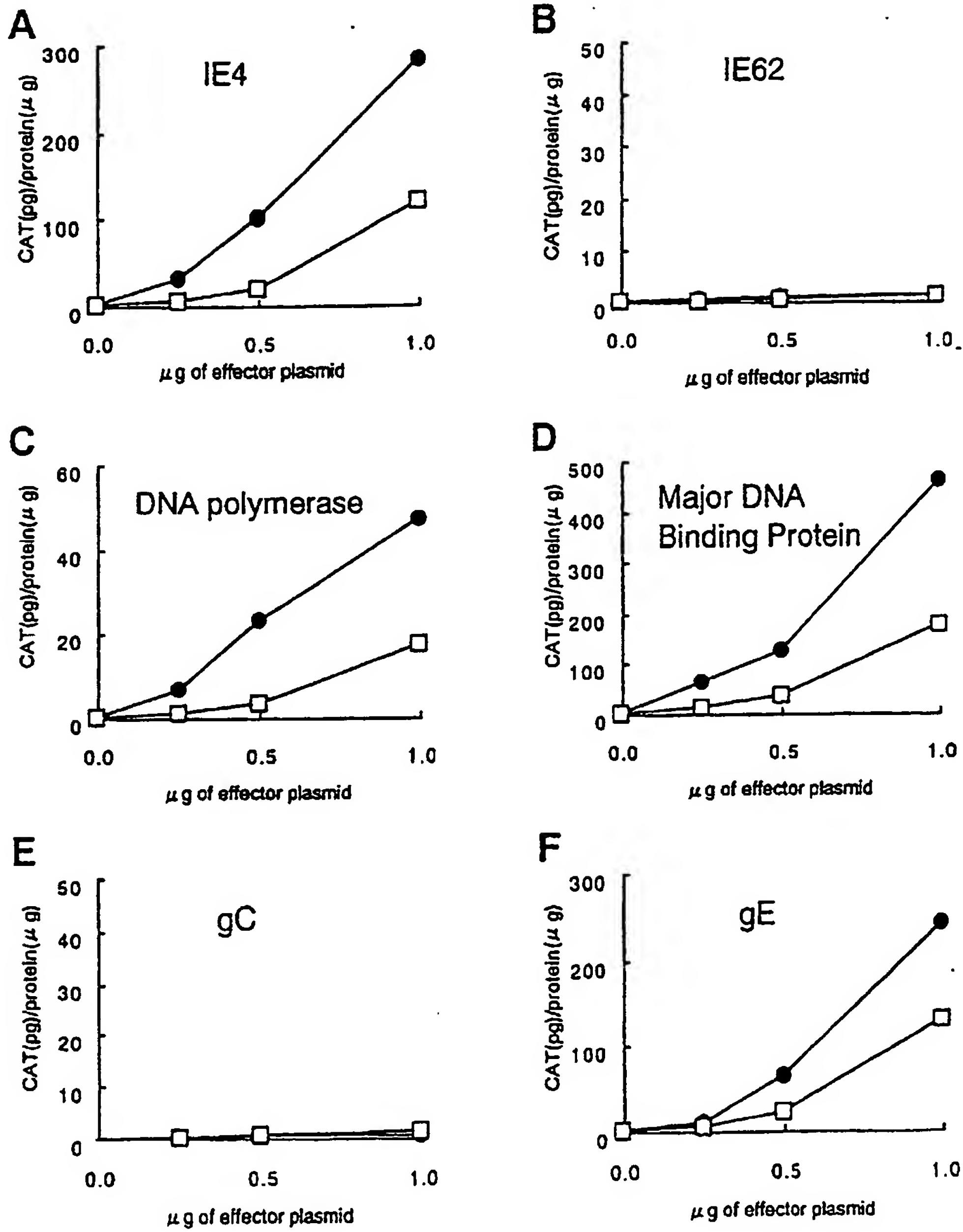


Fig. 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/05476

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl.⁷ C12N15/38, C12N5/06, C12N7/08, C12P21/08, C12Q1/68, C12Q1/70,
C07K14/04, C07K16/08, A61K39/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl.⁷ C12N15/38, C12N5/06, C12N7/08, C12P21/08, C12Q1/68, C12Q1/70,
C07K14/04, C07K16/08, A61K39/25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI (DIALOG), BIOSIS (DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, 510996, A1 (RES FOUND MICROBIAL DIS UNIV OSAKA), 28 October, 1992 (28.10.92)	1-4, 9, 15, 16
Y	& JP, 06-189752, A & AU, 659449, B & US, 5849476, A & CA, 2066998, A & TW, 210354, A	5-8
X	US, 3985615, A (RES FOUND MICROBIAL DIS UNIV OSAKA), 12 October, 1976 (12.10.76)	1-4, 9, 15, 16
Y	& JP, 53-041202, B & BE, 826568, A & FR, 2263786, A & GB, 1457382, A	5-8
Y	FORGHANI, B. et al. "Monoclonal antibody to immediate early protein encoded by varicella-zoster virus gene 62", Virus Res. (1990), vol.16, No.2, p195-210	5-8
A	WO, 97/43420, A1 (RES FOUND MICROBIAL DIS UNIV OSAKA), 20 November, 1997 (20.11.97)	10-14
A	& EP, 839911, A1 & CN, 1193351, A	
A	DAVISON, A.J. et al. "The complete DNA sequence of varicella -zoster virus", J.gen.Virol. (1986), vol.67, No.9, p1759-1816	10-14

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not
considered to be of particular relevance"E" earlier document but published on or after the international filing
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considered novel or cannot be considered to involve an inventive
step when the document is taken alone"Y" document of particular relevance; the claimed invention cannot be
considered to involve an inventive step when the document is
combined with one or more other such documents, such
combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 January, 2000 (24.01.00)Date of mailing of the international search report
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